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REVIEW

Review of recent literature on microneedle vaccine delivery technologies

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Abstract: Microneedles (MNs) have been developed as medical devices for enhanced and painless transdermal drug and vaccine delivery. MN-based vaccine application, unlike conventional intramuscular or subcutaneous application using hypodermic needles, delivers vaccine directly into skin, which is known to be an immunologically much more relevant vaccination site than underlying tissue. Vaccination using MN devices targets the skin's rich immune system, leading to better utilization of the antigen and resulting in superior immune response, often achieved using a lower vaccine dose than required by conventional delivery routes. However, despite the number of advantages and nearly four decades of research, the number of licensed MN-based vaccines remains limited to date. Nevertheless, it is to be expected that on the back of a number of recently developed scalable and robust MN-fabrication methods, more intensive translation into clinical practice will follow. Here, we review the current status and trends in research of MN-related vaccine delivery platforms, focusing on the most promising approaches and clinically relevant applications.

Keywords: microneedles, vaccine delivery, skin vaccination

Introduction

Most current human vaccines are still delivered using a hypodermic needle. With a few rare exceptions, such as oral polio vaccine or intranasal influenza vaccine, traditional intramuscular or subcutaneous injection is still the preferred route of application, even for novel vaccines. This is not due to a lack of alternative approaches – quite the contrary. A number of alternative needle-free vaccine delivery platforms have been suggested over the past few decades.[1] The long list includes edible vaccines,[2] various physical methods for delivery of DNA-based vaccines (gene gun, electroporation, ultrasound[1,3,4]), high-velocity powder and liquid-jet injection,[5,6] diffusion-based patches combined with skin abrasion/ablation[7–9] and chemical enhancers,[10] microneedles (MNs),[11] and others.[12] Many of these platforms are at least partially complementary in their potential applications. The choice of the optimal delivery method largely depends on the nature of the vaccine (protein, DNA, virus, etc), nature of the adjuvant, and formulation details. To date, the most commercially successful have been devices compatible with existing liquid or lyophilized formulations based on liquid-jet and microinjection/microneedle technologies.[13] Use of MN-based technologies for vaccine delivery has been attracting considerable interest lately, driven by both increasing knowledge of skin immunology and advances in microelectronics, which enable production of such micron-scale devices. The concept of submillimeter needle-shaped structures that would pierce through the outermost skin layers and increase their

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permeability several thousandfold but without stimulating the underlying nerve endings and causing pain was first conceptualized in 1976.[14] Early development of MNs was focused mainly on delivery of low-dose, low-molecular-weight drugs into the skin. In the last decade, the use of MNs as a vaccine delivery platform has gained significant interest, driven largely by recent efforts to develop novel, painless, and dose-sparing influenza vaccines.[11,15]

MN-assisted vaccine delivery has the potential to overcome many of the disadvantages of traditional needle-and-syringe delivery routes. Injections using hypodermic needles are invasive and painful, the vaccine delivered may have a less than ideal pharmacokinetic profile,[16] and result in intentional reuse and unintentional needle injuries, which may be responsible for over a half million deaths annually due to transmission of infectious diseases.[17] MN-based vaccine platforms may deliver the vaccine dose reproducibly, enhance the pharmacokinetic profile, improve the safety of the application, decrease the level of expertise needed for application, and eliminate the risks and costs of sharps waste disposal. In addition, there is a reasonable potential that for certain types of vaccines, the dose needed for effective immunization using MNs may be significantly lower than if delivered via conventional application routes. Intramuscular and to some extent intradermal injection of vaccine bypasses the upper layers of skin and the skin's immune system and delivers the vaccine into the immunologically less relevant areas, such as muscle and subcutaneous tissue. The skin, in contrast, is exceptionally rich in dermal dendritic cells and epidermal Langerhans cells, and provides efficient drainage to lymph nodes, thus making it a much more attractive site for vaccination.[18,19] Such improved utilization and better pharmacokinetic profile of antigen(s) delivered may result in dose-sparing, which may be especially relevant in the case of urgent need of a large quantity of vaccines, eg, in the case of unexpected pandemics.[20–26]

Vaccine delivery using microneedles: current status

Use of MN-based platforms for vaccination has been extensively studied using a large number of model antigens and clinically relevant vaccines (Table 1). To date, by far the most studied have been possibilities for using MN platforms for influenza vaccination. This is more a reflection of the general focus of global vaccine research in recent years rather than the particular suitability of influenza vaccines for MN delivery compared with other pathogens.

Most studies were animal models, and very few human trials reports are available.[45,48–51] It is generally accepted that major immunological findings obtained using appropriate animal models can in their essence be extrapolated to the expected results of future human studies.[92,93] However, certain results derived from animal studies of MNs have to be evaluated keeping in mind differences in the structure and elasticity of animal versus human skin. For example, mouse skin is known to be more elastic than human skin, making it more resistant to MN penetration.[43] If applicable, perhaps the best laboratory model for MN research is the use of human cadaver skin,[39,67] albeit availability and regulatory requirements may dissuade one from this approach.

Despite outstanding research-and-development efforts, most of the MN-vaccine projects are still in the proof-of-concept stage, with only a few pursued into clinical stages and even fewer resulting in licensed MN-based vaccines. This is partly due to the fact that a great amount of research in the MN field to date has been focused on the development of various MN platforms and resolving numerous related technical issues rather than exploring clinically relevant applications of MNs. Furthermore, a major limitation of most of the available MN platforms seems to be the dose problem. The amount of vaccine that can be effectively delivered using MN devices remains fairly low, roughly estimated at approximately 1 mg of dry content for a small MN array, which in many cases

Table 1 Vaccines and model antigens used in microneedle-based vaccine delivery trials

Proteins and inactivated viruses	Virus-like particles	Live viruses	Bacterial antigens	DNA
Influenza[23,24,26–52]	Influenza[22,64–69]	Adenovirus[54,71,72]	BCG[76]	Hepatitis B[84,85]
Ovalbumin[53–57]	HPV[70]	MVA[72,73]	Tetanus[32]	Hepatitis C[86]
BSA[58]		Measles[74]	Diphtheria[32,43,77–79]	Influenza[27,87,88]
Rotavirus[59]		Japanese encephalitis[75]	Botulism[80]	HIV[71]
HIV[60]			Malaria[32]	Smallpox[89]
Chikungunya virus[61]			*Yersinia pestis*[80,81]	Herpes simplex virus[90,91]
Rabies virus[62]			*Staphylococcus aureus*[80]	
Hepatitis B[63]			Anthrax[80,82,83]	

Abbreviations: BSA, bovine serum albumin; HPV, human papillomavirus; MVA, modified vaccinia virus Ankara; BCG, bacillus Calmette–Guérin; HIV, human immunodeficiency virus.

is insufficient to accommodate the required human vaccine dose.[11,94] An obvious way to increase the dosage is to enlarge the MN array. However, increasing the array size brings additional technical challenges, increases production costs, and makes application less convenient. The maximum MN patch reported to date is a 25 cm^2 array, containing approximately 18,000 individual MNs.[95]

The dose problem is likely to be the main technical challenge to be resolved before we see more MN-based vaccines entering clinical phases of development. Therefore, it may be expected that in the short run, development of MN-mediated vaccines will be focused mainly on low-dose and/or self-replicating vaccines, eg, tuberculosis vaccine or any of the live viral vaccines.

Microneedle platforms for vaccine delivery

Several diverse MN platforms have been developed to date, which can be divided into four groups based on their basic principle of operation (Figure 1). A great amount of technical skill and engineering ingenuity has been needed for the development of robust fabrication methods for such complex micron-scale devices. Especially challenging is the development of MN-fabrication methods that have the potential to be economically scaled up and installed to pharmaceutical standards. This is still a very lively field of research, with new technologies and approaches emerging frequently. As discussed earlier, there are still a lot of technical issues to be resolved before we see more examples of successful scaling up of laboratory setups to the industrial level and transfer into the clinical stage of development. Recent advances in most promising MN platforms are discussed below.

Solid microneedles

The simplest form of MN devices are solid MNs, and most of the early work on MN-assisted delivery of vaccines was done using this type of MN. Solid MNs are usually 70–800 μm long and arranged into one- or two-dimensional arrays, forming an MN patch. A large number of materials have been tested for fabrication of solid MNs, ranging from silicon and metal to nondegradable polymers and ceramics (for a review, see Kim et al[11]).

Solid MN patches can be used naked for skin pretreatment, and when inserted onto the skin and removed they open pores in the skin surface. Drug or vaccine applied onto treated skin surface diffuses into the skin through pores created by MN pretreatment.[73] This approach was used to immunize mice against diphtheria toxoid, giving satisfactory results, and influenza subunit vaccine, with less adequate response.[43] Better humoral and cellular immune responses against hepatitis B DNA-based vaccine applied topically after skin scraping using solid MNs in comparison with intramuscular or intradermal vaccination by injection were seen in mice.[85] Recombinant modified vaccinia virus Ankara (MVA) expressing a malaria antigen was administered to mice using a range of silicon MN patches with different MN height, density, patch area, and total pore volume. Interestingly, it was found that the design of MN patches significantly influenced

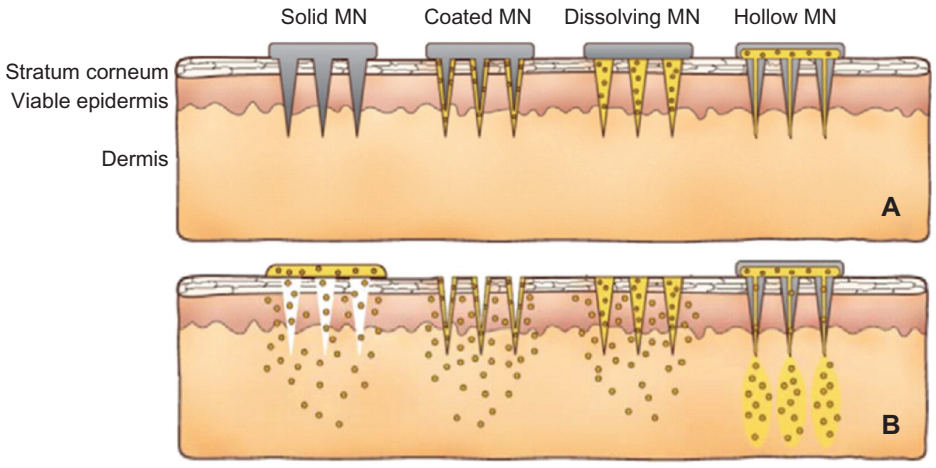

Figure 1 (**A** and **B**) Microneedle (MN)-based platforms for drug and vaccine delivery (reproduced with permission from Kim et al[11]). Solid MNs pierce through the outermost layers of the skin, leaving open pores (**A**) after which drug/vaccine is delivered into the skin (**B**). Solid MNs may be used for skin pretreatment, after which drug/vaccine is topically applied and left to diffuse into the skin through opened pores. An alternative way is to precoat the MN array with a formulation that dissolves off the MN patch upon contact with the skin (coated MN). Dissolvable MNs contain active ingredient incorporated into water-soluble biodegradable matrix that dissolves on contact with the skin and releases drug/vaccine (dissolving MN). Hollow MNs are used for direct injection of liquid formulation into the skin (hollow MN).

the magnitude and memory of vaccine-induced $CD8^+$ T-cell responses and could be optimized for the induction of desired immune responses. Also, unlike administration using hypodermic needles, MN-mediated vaccination did not induce inflammatory responses at the site of immunization or in draining lymph nodes.[73]

The other approach in using solid MNs for vaccine delivery is precoating of MN patches with water-soluble vaccine formulation before insertion into the skin. After application of such coated MN patches the vaccine is dissolved and released off the patch and into the skin, after which the patch is removed.

Precoating of solid MN arrays with a stable vaccine formulation incorporates drug and delivery device into a single delivery system, thus simplifying application. Techniques for precoating of MN devices include coating by repeated immersion,[96–98] simple dip-coating,[26,42,98] gas-jet dry-coating,[99,100] coating using aerosolization,[101] and spray-coating.[72,102,103]

Vaccines coated onto solid MN patches include inactivated influenza[26,47,52,100,104] and Chikungunya virus vaccines,[61] virus-like particle-based influenza[69] and human papillomavirus vaccines,[70] hepatitis C,[86] West Nile virus[61] and herpes simplex virus 2 DNA vaccines,[90,91] and live MVA and adenoviruses.[72] In general, results using MN devices were comparable or superior to those obtained using intradermal or intramuscular routes of application.

Based on the declining proportion of publications describing the use of solid MN vaccine delivery devices in comparison with dissolvable and hollow MN platforms, it seems that these other options may start to dominate the field in upcoming years. However, some solid MN devices for vaccine delivery have shown promising results in the preclinical stage, with clinical trials announced for 2013.[47,105,106] In addition, most current fabrication methods for dissolvable MNs rely on usage of polydimethylsiloxane molds, which are cast using solid MN arrays. Therefore, although the work on direct use of solid MN arrays for vaccination may be in slow decline, fabrication and design of solid MN arrays is likely to remain a matter of intensive research.

Hollow microneedles

Hollow MNs are miniature needles used for direct application of liquid formulation into skin. There are two types of hollow MN designs. One uses a single MN, thus resembling a miniature conventional hypodermic needle. More often, hollow MNs are arranged into arrays, enabling simultaneous application of a vaccine formulation over a wider area of skin. This may allow not only faster application of the vaccine but higher bioavailability and antigen utilization as well, since a larger application area increases the chances of lymphatic uptake of antigens.[107]

The use of hollow MNs has the great advantage of enabling the use of liquid-vaccine formulations rather than vaccines in a dried form, as required by other MN platforms. Some vaccines cannot be presented in a dried form or may lose activity upon conversion into a dry state, making the use of hollow MNs the preferred choice. On the other hand, hollow MN devices usually require coupling with a syringe or other liquid container and perhaps assistance of trained personnel for application.[108]

To date, an influenza vaccine based on a single hollow MN system has been the most successful commercial application among the vaccine MN-delivery systems.[109,110] It is, however, debatable whether this system, marketed as BD Soluvia Microinjection System, is an MN or rather a short-needle system, given its 1.5 mm length compared with other MN devices that are regularly well below 1 mm in length.[111] In one study, vaccination using such a single hollow MN device resulted in superior immunogenicity among elderly, which is particularly relevant, as morbidity and mortality from seasonal influenza is the highest in this population.[50] Also important is the significant dose-sparing effect achieved using the MN device compared with the intramuscular vaccination route.[45,49] The dose of 15 μg of hemagglutinin required for the intramuscular route was reduced to 9 μg for the MN device, giving superior immune response.[29,49]

The potential of using hollow MN arrays for vaccine delivery was also demonstrated by immunization with ovalbumin and DNA-encoding reporter genes.[112] Further, few clinically relevant vaccines have been successfully delivered using hollow MN devices in animal models. Vaccines against anthrax[83] and Japanese encephalitis[75] were successfully delivered and shown to be safe and efficacious.

Dissolvable microneedles

Most recently, a very promising approach for MN-mediated vaccine delivery was based on the use of dissolvable MN arrays. This platform first appeared in 2005,[113] and has gained significant interest to date. The main idea behind dissolvable MN platforms is incorporation of vaccine into rigid polymeric or sugar MNs (for a review of fabrication methods, see Kim et al[11]). Upon insertion of dissolvable MNs into the skin, water evaporating from the opened pores dissolves the MN matrix and releases the vaccine, which then diffuses

easily into the skin. Materials used in the fabrication of the dissolvable MN matrix have to be inert, safe, water-soluble, sufficiently hard in dried form, and compatible with vaccine components.

Interest in dissolvable MNs seems to have increased in recent years, driven by the development of a number of dissolvable MN-production methods. These are mostly mold-based techniques, and include various casting, injection-molding, hot-embossing, diffusion-into-mold, spraying-into-mold, and similar techniques.[111,114,115] Materials tested for suitability in the preparation of dissolvable MNs are indeed numerous. Most of these are various polymers or sugars. Polymers include carboxymethyl cellulose, chondroitin sulfate, polyvinylpyrrolidone, polyvinyl alcohol, poly(lactic-*co*-glycolic acid), dextran, and dextrin, while the sugars most used are trehalose, maltose, sucrose, and galactose (for reviews, see Prausnitz et al[108] and Donnelly et al[111]).

The advantages of dissolvable MNs over other MN platforms are claimed to be cost-effectiveness, biodegradability, robustness, and scalability.[111,114,116,117] Among the disadvantages, two issues may be problematic for certain types of vaccines. One is the already-discussed problem of the dose. The amount of vaccine that can be mixed with matrix components without compromising hardness and rigidity upon drying is fairly limited. Hence, the total amount of vaccine that can be accommodated in an average-size dissolvable MN patch is measured on the milligram scale, which may be insufficient for many conventional human vaccines.[108] The other problem related to incorporation of vaccine into a dry polymer/sugar matrix is stability of active component(s). Traditionally, many vaccines are stabilized in a dry form by freeze-drying. However, unlike structurally very fragile freeze-dried cake, the matrix of dissolvable MNs has to retain rigidity and hardness to enable penetration into the skin. The properties of such polymer/sugar matrix are very much different from those of freeze-dried forms, and finding a suitable combination of compatible and suitable matrix components may be problematic. Generally, the stability of protein antigens, including inactivated viruses, may be well preserved in certain dissolvable MN platforms,[118] while the stability of live viruses is likely to be more challenging.[114]

Examples of successful embedding and delivery of vaccines using dissolvable MN platforms include plasmid-encoding hepatitis C antigens,[86] proteins,[53–55,104,119] inactivated influenza viruses,[53,104,118,120] and live adeno- and MVA viruses.[114] To date, there have been no reports on human trials using dissolvable MN vaccines. However, given the advantages of dissolvable MNs, in particular industrial scalability and cost of production of dissolvable MN patches, encouraging results of preliminary animal studies, and liveliness in discovery of novel fabrication methods, it is likely that research efforts in this field will bring forth some vaccine products in upcoming years.

Prospects of microneedle-based vaccine delivery technologies

Research into MN devices is in its fourth decade already. Given the time span and amount of published data, it is somewhat surprising to see the relatively small number of commercial MN-based pharmaceutical products, and MN-based vaccines in particular. The main reason is probably the fact that until recently, fabrication methods for MN arrays were not mature enough to be the basis of a robust and reproducible industrial process, needed in the pharmaceutical environment. Hence, the interest of the pharmaceutical industry in the research of MN devices was somewhat limited. Recently, however, with the introduction of microelectronic- and laser-based state-of-the-art methods into MN research, it has become possible to develop production methods that have the potential of scalability and translation of laboratory settings into a good-manufacturing-practice environment. Therefore, it can be expected that enormous efforts invested in design and fabrication methods of MN devices will finally start giving more clinically relevant results in this decade.

The other important reason that translation of MN-based vaccines into clinical use is still somewhat slow is the sole fact that in most cases, despite all the aforementioned disadvantages, traditional intramuscular or subcutaneous application of a vaccine results in a sufficient and reliable immune protection. The 100-year-long paradigm of needle- and syringe-based application of vaccines is simply not easy to put into question. However, the fact that current needle-and-syringe methods work fine may slow down an easy penetration of MN-based alternatives, but with further technological improvements, accumulation of data on efficacy and safety, and paying more attention to the patient's comfort, we are likely to see the introduction of a number of MN-based vaccines by the end of this decade.

To conclude, research on MN devices for vaccine delivery was until recently more about solving the design and fabrication issues, while now focus is swiftly changing to the use and application of MNs for delivery of clinically relevant vaccines. It is therefore to be expected that vaccination will soon start becoming a needle-free practice.

Disclosure

The author reports no conflicts of interest in this work.

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